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# Thin-film silica sol-gels doped with ion responsive fluorescent lipid bilayers

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## ABSTRACT

A metal ion sensitive, fluorescent lipid-bilayer material (5% PSIDA/DSPC) was successfully immobilized in a silica matrix using a tetramethoxysilane (TMOS) sol-gel procedure. The sol-gel immobilization method was quantitative in the entrapment of self-assembled lipid-bilayers and yielded thin films for facile configuration to optical fiber platforms. The silica matrix was compatible with the solvent sensitive lipid bilayers and provided physical stabilization as well as biological protection. Immobilization in the silica sol-gel produced an added benefit of improving the bilayer's metal ion sensitivity by up to two orders of magnitude. This enhanced performance was attributed to a preconcentrator effect from the anionic surface of the silica matrix. Thin gels (193 micron thickness) were coupled to a bifurcated fiber optic bundle to produce a metal ion sensor probe. Response times of 10 – 15 minutes to 0.1 M $CuCl_2$ were realized with complete regeneration of the sensor using an ethylenediaminetetraacetic acid (EDTA) solution.

**Keywords:** metal ion sensor, fluorescence, fiber optics, sol-gel materials, lipid bilayers, TMOS, ion recognition

## 1. INTRODUCTION

New synthetic sensor materials based on lipid molecular assemblies have recently been developed to detect low levels of metal ions,[1,2] polypeptides,[3] proteins,[4] virus particles,[5,6] and toxins.[7,8,9] The lipid bilayers are functionalized with synthetic and biological receptors and generate easily observable fluorescent or colorimetric responses via structural changes in the membrane via host-guest interaction at the membrane surface. By mimicking the cellular membrane surface the lipid bilayers offer a unique environment for host-guest interactions and direct coupling of those interactions to a defined and molecularly dynamic material. These unique sensor materials are self-assembled and can be held together by forces that range from weak van der Waals interactions to covalent bonds. The ability of these molecular assemblies to reorganize in response to molecular recognition events at the membrane surface is responsible for the highly sensitive and rapid response observed.

The coupling of these lipid membrane materials to optical platforms would create new sensor systems for the facile and continuous monitoring of aqueous streams or industrial processes. However, the immobilization of lipid bilayers to a solid surface remains problematic for these fragile molecular assemblies. Langmuir-Blodgett multi-layer films[10] and cast multi-bilayer structures[11] can be coupled to solid surfaces, but are unstable in solution and tend to exfoliate from the surface with time. Additionally, the first layer of a multi-layer film would mask membrane receptors beneath to solution and analyte exposure, thus greatly impeding sensor response. Vesicle structures,[12] on the other hand, offer excellent receptor exposure to the solution phase and are easy to prepare, handle, and store. Immobilization of these materials into solid, organic based matrices has yielded some successes.[13,14,15] However, some shortcomings remain with entrapment volume, leakage, labor, limitations in types of lipid-based structures that can be entrapped, and resistance to biological attack.

A new method to immobilize vesicular lipid bilayers has been developed in our laboratory that uses a metal oxide gel to quantitatively entrap lipid-based sensor materials.[16] The sol-gel process used was mild to the bilayers and could be used to form various structures, such as monoliths or thin films. Entrapped bilayers are physically stable in the gel. No lipid leakage was observed over several months for gels placed in a flowing aqueous stream. An additional benefit of immobilizing metal sensitive materials in the metal oxide gels was an observed enhancement in sensitivity to solution-phase metal ions. Herein, we report on the material characteristics of these unique sol-gel composites and examine relationships that may exist with their optical response to aqueous phase divalent metal ions. A brief description of the lipid membrane sensor material is also presented followed by the procedures for sol-gel entrapment.

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## 2. LIPID BILAYERS AND SOL-GEL IMMOBILIZATION

Functionalized synthetic lipid bilayers were immobilized in a robust silica matrix using the facile technique described below. Figure 1 illustrates the process of silica sol-gel formation around a bilayer vesicle. The process conditions were mild producing no detectable changes to the bilayer structure. The resultant transparent and porous material offered ideal optical and good transport properties for the detection of aqueous phase analytes.

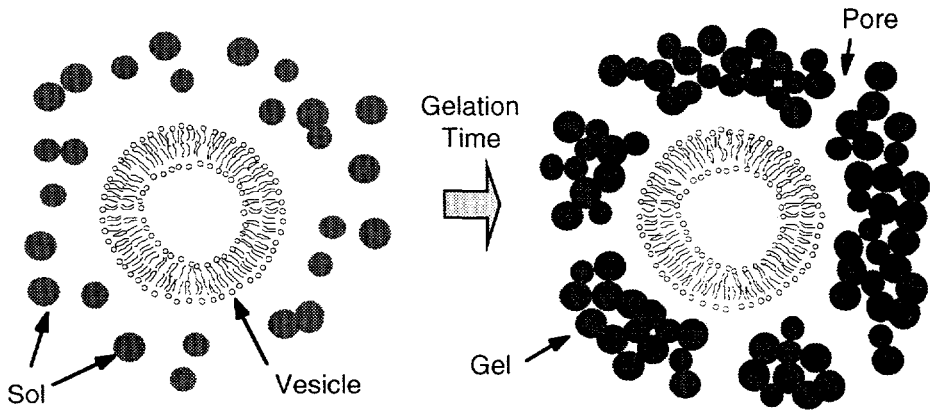


Figure 1. Illustration of the sol-gel immobilization of a lipid bilayer vesicle.

In this section, a brief description of the preparation of a functionalized lipid-bilayer and the mechanism of the optical response to metal ion recognition will be given. The sol-gel entrapment procedure will then be described for the preparation of both monoliths and thin film materials. Characterization of the resultant materials regarding porosity and structure will then be presented followed by an examination of the stability of the bilayers in the silica matrix.

### 2.1. Metal Ion Sensitive Lipid Bilayer

The metal ion sensitive bilayer was prepared via synthetic routes using a totally synthetic receptor lipid and a commercially available, biologically derived lipid. Both lipids are shown in Figure 2. The synthetic lipid, named PSIDA, was designed to mimic the structure of biological lipids, such as that of the matrix lipid distearylphosphatidylcholine (DSPC). Several modifications were made, however, which include the metal ion receptor at the headgroup position, pyrene fluorophore at the tail, and ether linkages near the backbone for added chemical stability. A detailed description of the synthetic steps used to prepare PSIDA is given elsewhere (see reference 3).

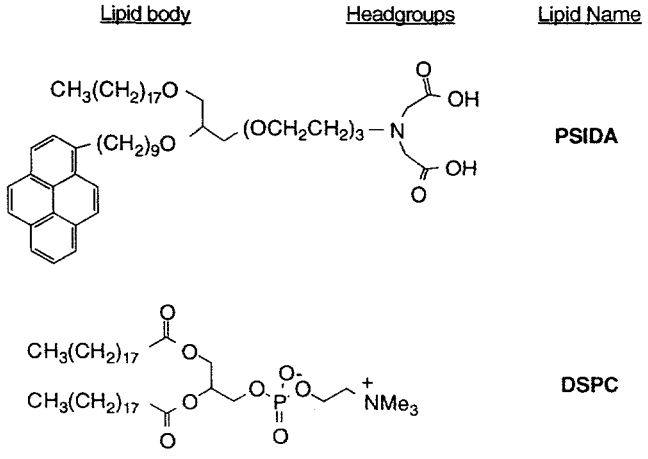


Figure 2. Molecular structures of PSIDA and DSPC.

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The pyrene fluorophore was used as an optical probe to monitor aggregational changes of the PSIDA in response to metal ion recognition at the receptor. Through an interplay of physical and chemical forces, the aggregational properties of the PSIDA lipids can be controlled by the addition or removal of selected metal ions. Bilayers of 5 mole % PSIDA/DSPC are initially phase separated with the liquid phase PSIDA lipids aggregated into pools in the midst of crystalline domains of DSPC. The high local concentration of pyrene gives a bilayer fluorescence spectrum having a strong pyrene excimer emission ($\lambda_{max}$ = 470 nm) relative to the monomer emission ($\lambda_{max}$ = 376 nm). Since the pyrene probe is sensitive to any local concentration changes in the bilayer and the concentration of PSIDA in the bilayer is constant, any changes in the ratio of intensities from the excimer to monomer emissions are a reflection of changes in the aggregational state of the PSIDA lipid. Figure 3 shows an example of the fluorescence response of the 5% PSIDA/DSPC bilayers to various concentrations of $MnCl_2$. By plotting the excimer to monomer intensities (E/M) against the concentration of various metal ions we can observe a high selectivity and sensitivity for $Cu^{2+}$ of the 5% PSIDA/DSPC bilayer material, as seen in Figure 4.

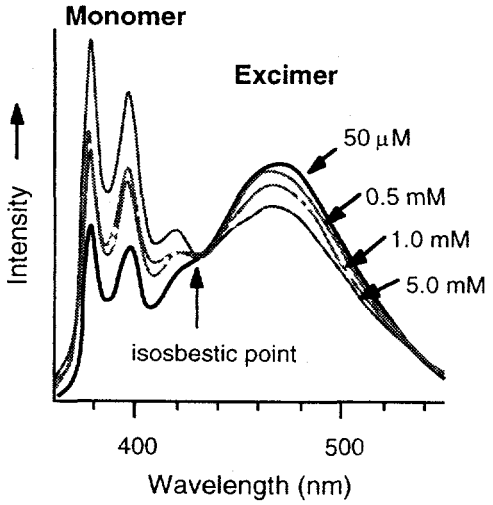


Figure 3. Fluorescence spectra[17] of 5% PSIDA/DSPC bilayers in aqueous MOPS buffer solution with varying amounts of $MnCl_2$.

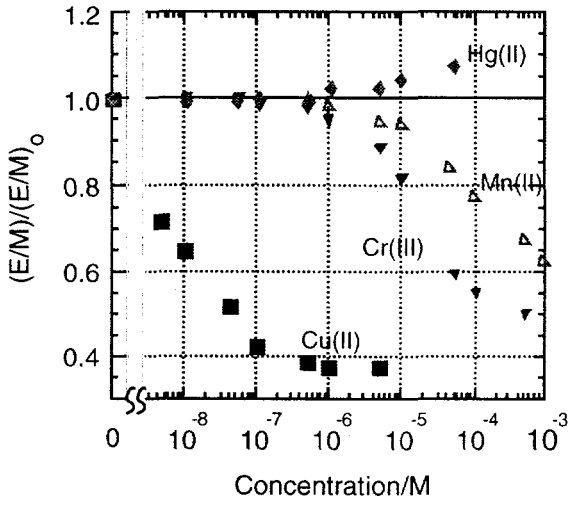


Figure 4. Graph of normalized E/M response of 5% PSIDA/DSPC bilayers vs. concentration to several metal ions.

The fluorescence response of the functionalized lipid bilayer is specific to metal ion chelation at the membrane surface. Through various experiments we have shown that the optical response was not associated with any metal ion quenching phenomena or non-specific binding interactions with the membrane surface. Instead, the synthetic membrane receptor selectively binds divalent and trivalent metal ions forming a cationic lipid headgroup. The charge repulsion of the metal ion complexed headgroups disperses the PSIDA lipids into the DSPC matrix, thereby reducing the pyrene local concentration and a subsequent decrease in the E/M value. Figure 5 summarizes the change in molecular aggregation of the lipid membrane induced by metal ion recognition. The membrane receptor of the functionalized lipid bilayers can be tailored to selectively detect other heavy metal ions, such as $Hg^{2+}$ and $Pb^{2+}$.[2,18]

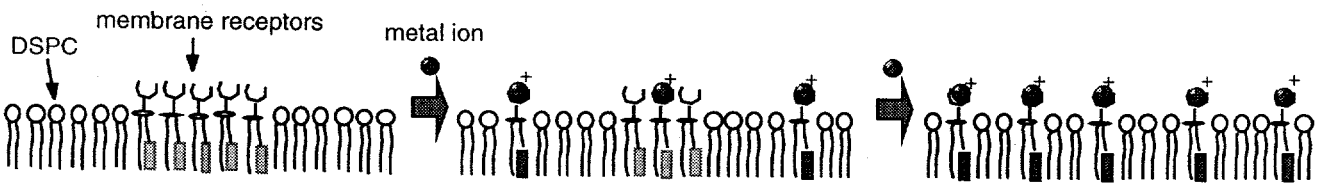


Figure 5. Illustration of metal ion induced molecular reorganization in outer half of the lipid bilayer. The receptor lipids (e.g., PSIDA) are initially aggregated (far left). Binding of metal ion creates charged headgroups and repulsion between lipids dispersing the receptors into the matrix (middle). At high metal concentration all receptors are occupied and dispersion of receptors is at a maximum (far right).

## 2.2. Lipid Bilayer Entrapment in TMOS Sol-gels

The sol-gel method used for the entrapment of lipid-bilayers is an adaptation of a general procedure for the entrapment of biological materials, such as proteins.[19,20] The sol is mostly aqueous and brought to biological pH (ca. 7.4) prior to addition of the lipid bilayers. Gelation occurs rapidly at room temperature but can be retarded by cooling in an ice bath. Gels were formed as both monoliths and thin films. As monoliths the gels were transparent, hard, and brittle. Thin films were prepared using a polypropylene mesh as a scaffold giving the material pliability and strength.

The silica sol was prepared by sonicating 15.25 g of tetramethylorthosilicate (TMOS), 3.35 g of deionized water, and 0.22 mL of 0.04 N aqueous hydrochloric acid in a chilled water bath for approximately 20 minutes. To 5.0 mL of the homogeneous sol was added 7.5 mL of the 5% PSIDA/DSPC lipid bilayer solution chilled in an ice bath. The mixture was rapidly stirred then immediately poured into polystyrene cuvettes for the preparation of monoliths (18 mm x 10 mm x 5 mm) or formed as a thin film using an open pore mesh. For the thin film preparation, sol was poured over a polypropylene mesh (Spectramesh: mesh opening 149 μm, open area 34%, thickness 193 μm) then sandwiched between two pieces of polyethylene film for several hours. The mesh acts as a scaffold to support gels of 193 micron thickness. The total lipid concentration was 0.2 mM for the thin films and 20 μM for monoliths. All gels were aged in MOPS buffer solution (0.02 M 4-morpholinopropanesulfonic acid, 0.10 M NaCl, pH adjusted to 7.4 with 10% aqueous NaOH) for several days prior to use.

Characterization of the silica gel matrices of freshly prepared and aged materials were conducted using scanning electron micrographs (SEM) and porosimetry measurements. Since the sensor materials perform as wet gels, a method to remove the aqueous phase without loss of material size or shape or alteration of the matrix was required. Super critical carbon dioxide ($SCCO_2$) extraction provided an excellent means to remove the aqueous phase under mild conditions with no chemical alteration of the surface. Porosimetry data of the resultant aerogels are shown in Table 1.

TMOS gels prepared with the 5% PSIDA/DSPC bilayers were examined in their initial state and after two months of aging in MOPS buffer solution. From the weight of the initial gel we can calculate that the TMOS from the sol is quantitatively incorporated into the gel. Aging in buffered water slightly decreases the overall dimensions and weight (i.e., $SiO_2$ content) of the gel by ca. 10%. Hydrolysis of the methylsiloxanes to produce silanols would account for a good portion of the weight loss. More significantly, aging greatly affects the porous structure of the silica gel. A decrease of over 50% in surface area is coincident with a 58% increase in pore diameter. Soaking of the gel in aqueous solution at pH 7.4 promotes further condensation and crosslinking of the silica matrix causing syneresis of the gel as well as a collapse of small pores and widening of larger ones.[21] The result is a gel with coarse structure. These matrix changes are coincident with fluorescence spectral data of the entrapped 5% PSIDA/DSPC lipid bilayers, discussed in the next section. Scanning electron micrographs (SEM) of the initial and two month aged gels are shown in Figure 6A and 6B, respectively. The coarse structure of the aged gel compared to the initial gel is consistent with the porosimetry data of Table 1.

**Table 1**. Pore Data of $SSCO_2$ Extracted Gel Monoliths

| TMOS gel | % decrease in width | Weight (mg) | Surface area ($m^2/g$) | Pore volume (cc/g) | Pore size (Å) |
|---|---|---|---|---|---|
| Initial | 0 | 119 | 1024 | 4.63 | 181 |
| Aged in MOPS buffer | 8 | 102 | 471 | 5.08 | 431 |

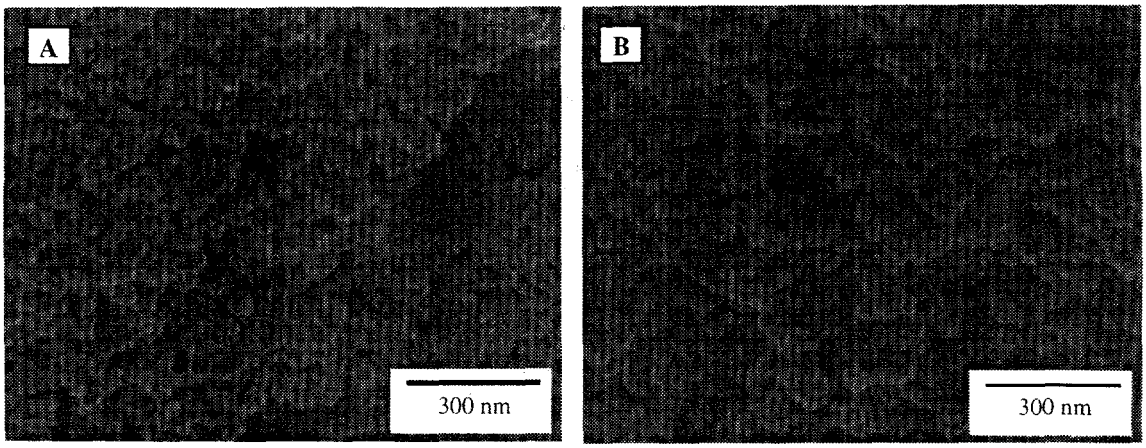


Figure 6. SEM of $SSCO_2$ extracted 5% PSIDA/DSPC-TMOS gels in the initial state (A) and after two months of aging (B) in MOPS buffer solution at pH 7.4.

### 2.3. Stability of Entrapped Bilayers

The sol-gel method provides an excellent method to quantitatively entrap lipid bilayer assemblies. The bilayers are stable in the matrix and exhibit no lipid leakage even after six months in a flowing aqueous stream. Complete removal of the entrapped lipids was readily achieved by extracting the gels with a 50% $CH_2Cl_2$/methanol solvent mixture. The silica matrix appears to be made up of a porous network connected to larger cavities that contains the lipid aggregates (ca. 300 Å diameter). The porous network must then consist of channels with diameters of smaller dimensions than the lipid bilayers to inhibit their transport out of the gel, yet large enough to allow facile passage of the lipid molecules (< 10 Å cross section width).

Entrapment of the bilayers in the silica gel has an advantage over organic based matrices in its ability to resist biological invasion and growth. The bilayers themselves are prone to microbial digestion. One of the requisites of the matrix material, then, is to provide a physical barrier of protection to biological attack. Matrices prepared from 5% agar gave clear, robust gels, but in a matter of days were overcome by microbial growth that extinguished bilayer fluorescence. For the TMOS gels, no loss of fluorescence or any sign of biological growth was observed even after several months of sitting in MOPS buffer solution. The silica matrix is not only a poor medium for biological growth but the pore dimensions may also inhibit the transport of bacteria (micron size) and single- to multi-celled animals. As a biological sensor this may provide a perm-selective barrier for the detection of small molecule toxins or virus particles in a biological soup.

An important feature of the lipid bilayer immobilization is the stabilization of the structures and inhibition of bilayer flocculation. Lipid vesicles in solution are often subject to flocculation with aging, binding of analytes on the membrane surface, temperature changes, and unfavorable solvent conditions. Metal ion recognition of the bilayers in free solution sometimes produces complicated response curves, which is believed to be due to light scattering effects from aggregate formation. Immobilized bilayers, on the other hand, yield improved linearity in response to metal ion concentration. Further discussion on these experiments can be found in the next section.

Another good example of the stabilization of the bilayers provided by immobilization in the silica gel is presented in the following temperature experiment (Figure 7). In the ideal case, increases in temperature result in an increased collision rate of lipids in the bilayer and a subsequent rise in fluorescence E/M values.[22] Upon reaching the melting point temperature for the matrix lipid the E/M value will drop, due to increased lipid mixing, followed again by increasing E/M with increasing temperature. Such ideal behavior was not observed with 5% PSIDA/DSPC bilayers in free solution due to flocculation and precipitation. The immobilized bilayers, in contrast, responded to temperature with ideal, and reproducible, fluorescence behavior.

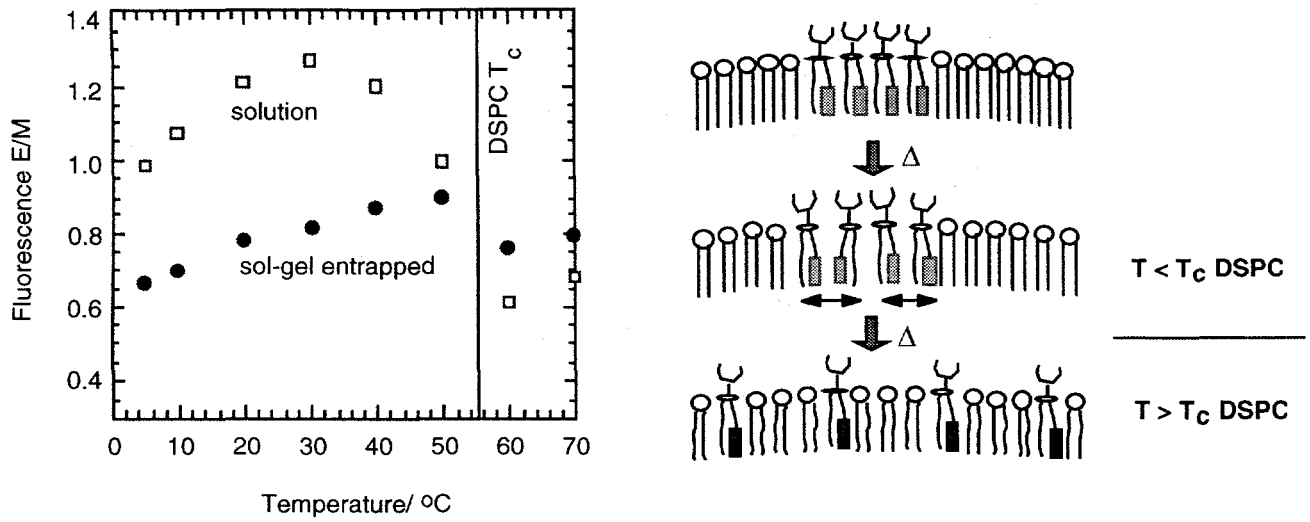


Figure 7. Temperature experiments with 5% PSIDA/DSPC as free solution aggregates and as immobilized in the silica matrix. Fluorescence E/M values are plot against temperature on the left side. On the right is an illustration of the changing lipid dynamics at various temperatures.

## 3. METAL ION RESPONSE OF FLUORESCENT GELS

Sol-gel immobilized 5% PSIDA/DSPC lipid bilayers exhibited excellent fluorescence response to metal ions in solution. The silica matrix does, however, influence the fluorescence properties of the lipid bilayer. In this section we will examine how the gel matrix affects the lipid bilayer's structure, metal ion sensitivity and selectivity, response time, and recycling ability.

### 3.1. Fluorescence Spectral Properties

Equilibration, or aging, of the silica sol-gel material in aqueous buffer solution, at pH 7.4, produces a change in fluorescence properties of the lipid bilayers. Both the overall fluorescence intensity and E/M ratio decrease with time, finally reaching constant values after several days. Figure 8 shows fluorescence spectra of an unaged and a gel aged for 5 days in MOPS buffer solution. The sol-gel process itself does not affect the fluorescence properties of the 5% PSIDA/DSPC bilayers. Identical spectra were obtained with equivalent concentrations of free and immobilized lipid bilayers in unaged gels suggesting that the sol-gel process is completely compatible with the bilayers. We have found in separate studies that the fluorescence properties of the 5% PSIDA/DSPC bilayer is unaffected by methanol at concentrations below 10% v/v. Thus, the amount produced by the TMOS sol-gel process must fall somewhere below this threshold.

Aging of the gel in buffered water: 1) removes the methanol produced from the TMOS sol-gel process, 2) hydrolyzes Si-$OCH_3$ groups and subsequently create a silicate surface, and 3) furthers Si-O-Si crosslinking in the gel.[21] First of all, removal of the methanol produced from the initial gelation process will not affect the fluorescence properties of the bilayers, as discussed previously. Hydrolysis and condensation of the silica gel will, however, alter steric constraints as well as the electrostatic field near the bilayers. The pore data from Table 1 find that the silica matrix collapses during aging going from a surface area of 1024 $m^2/g$ to 471 $m^2/g$ with a concomitant increase in pore size. By compressing the bilayer through a collapse of the matrix, the bilayer's hydrophobic interior may be compromised allowing water molecules near the pyrene fluorophore and some quenching to occur. This could explain the loss in fluorescence intensity with time of aging. Another possible cause of fluorescence intensity loss might be due to the formation of regions in the gel that scatter light thus decreasing light transmittance in to and out of the gel.

Interaction of the silanol surface of the gel with the lipid bilayer may play a role in the bilayer's observed decrease in fluorescence E/M. At pH 7.4, the silica surface should be largely deprotonated.[23] How such a surface will interact with an ionizable headgroup, like iminodiacetic acid,[24] is not understood. However, a pH study found that the sol-gel-immobilized 5% PSIDA/DSPC bilayers gave a pH dependent E/M profile with the highest value occurring at pH 10. In previous pH

studies of bilayers in free solution we have found that the highest E/M values occur between pH 7 – 8, corresponding to the zwitterionic form of the iminodiacetic acid group at the membrane surface. These results suggest that the silica matrix decreases the $pK_a$ of the lipid membrane surface by 2 – 3 pH units, forming cationic iminodiacetic acid headgroups at pH 7.4 that disperse PSIDA lipids and decreases the fluorescence E/M.

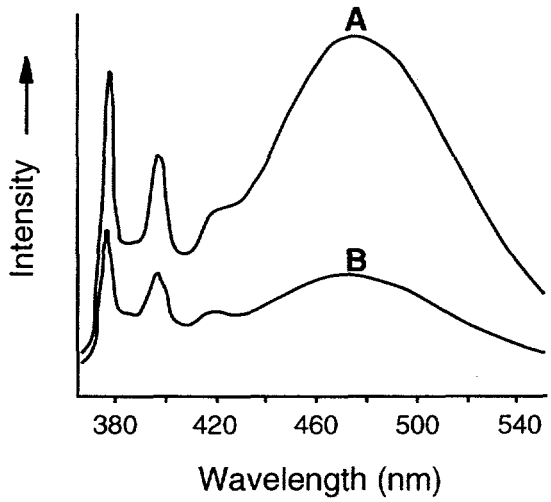


Figure 8. Fluorescence spectra of TMOS sol-gel immobilized 5% PSIDA/DSPC bilayers (A) unaged and (B) after aging in MOPS buffer solution, pH 7.4, for five days.

In spite of the above-mentioned changes in fluorescence properties of the sol-gel immobilized 5% PSIDA/DSPC lipid bilayers, their metal ion response is maintained. In Figure 9 is a photograph of two sol-gel monoliths, one unexposed and another exposed to 1.0 μM concentration of $CuCl_2$. The figure, which shows a change in brightness of the gel upon metal ion exposure, is actually a fluorescent color change from bluish-green of the unexposed gel to bluish-indigo of the exposed gel. Plots of E/M vs. metal ion concentration of sol-gel entrapped 5% PSIDA/DSPC compared against the bilayers in free solution revealed a significant improvement in metal ion sensitivity for the immobilized sensor materials. Figures 10 and 11 are E/M vs. metal ion concentration plots from the sol-gel monoliths and free solution bilayers responding to $Cu^{2+}$, $Mn^{2+}$, $Co^{2+}$, and $Ni^{2+}$. Enhancements in sensitivity of between 4 – 100 fold are observed. It is believed that the anionic surface of the silica matrix (vide supra) acts like a preconcentrator soaking in cationic metals from solution and increasing the local metal concentration near the bilayers. The same analyte/matrix interaction is also believed to be responsible for the slow transport rates observed for the metal ions. Fluorescence studies of the gel monoliths found that cations were up to 20 times slower through the gel compared to anionic EDTA or organic solvents.[16] The response curves of the immobilized bilayers also gave better linear response to metal ion concentrations. Free solution bilayers responding to some metal ions often produce complicated response curves that can be difficult to reproduce due to light scattering artifacts from bilayer aggregation induced by metal ion presence. Immobilization prohibits the aggregation of lipid bilayers and only the fluorescence response can be observed.

Figure 9. Photographs of TMOS sol-gel immobilized 5% PSIDA/DSPC (A) before and (B) after exposure to 1 μM $CuCl_2$ solution at neutral pH.

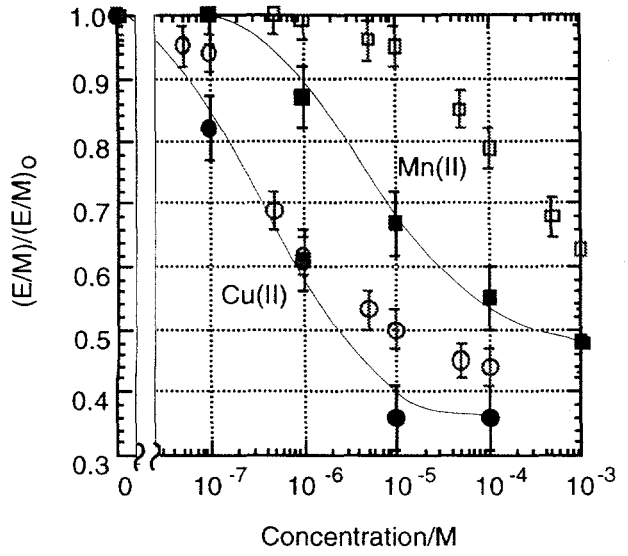


Figure 10. E/M response curves of sol-gel immobilized (filled symbols) and free solution (open symbols) 5% PSIDA/DSPC bilayers against $CuCl_2$ and $MnCl_2$. Lines are drawn as visual aids.

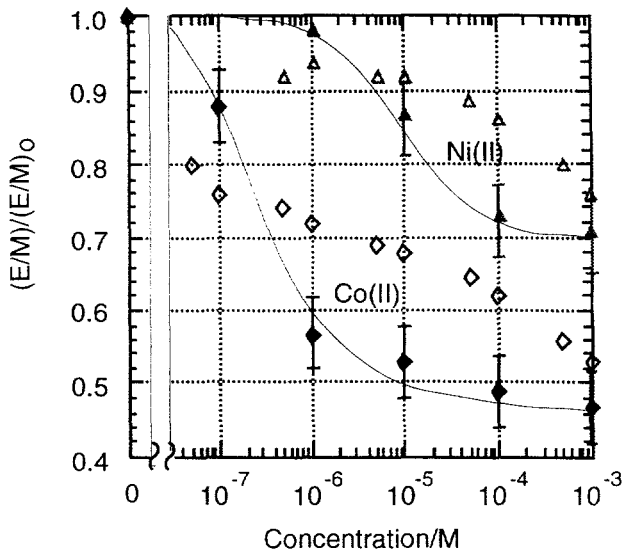


Figure 11. E/M response curves of sol-gel immobilized (filled symbols) and free solution (open symbols) 5% PSIDA/DSPC bilayers against $CoCl_2$ and $NiCl_2$. Lines are drawn as visual aids.

### 3.2. Thin Films on Optical Fibers

Thin films are being developed for coupling to optical fibers in an effort to improve the sensor response times of the sol-gel-immobilized bilayers. A 193 micron thin film of the TMOS sol-gel-immobilized 5% PSIDA/DSPC bilayers was prepared as described in Section 2. The films were fashioned into discs and configured to the distal tip of a bifurcated fiber optic bundle. The schematic of this probe design is shown in Figure 12. A dramatic 170-fold enhancement of response time was realized with the thinner gel compared to the 5 mm thick monolith. Equilibration times in excess of 24 with the monoliths at 0.1 mM $CuCl_2$ have now been reduced to approximately 10 - 15 minutes with the thin gels. The fluorescence response of the sensor probe to 0.1 mM $CuCl_2$ and recycling with 1.0 mM EDTA is shown in Figure 13, performed over several cycles.

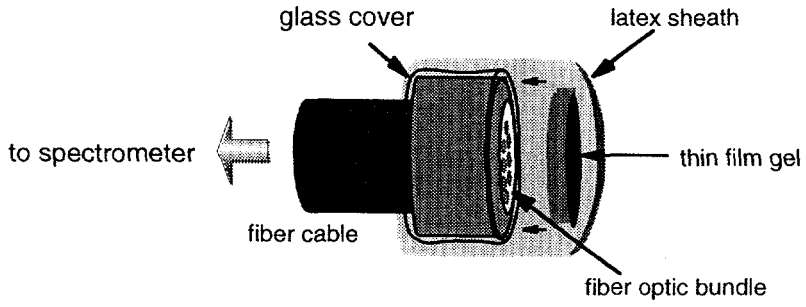


Figure 12. Schematic of the thin film coupling to bifurcated fiber optic bundle.

We are currently developing methods to couple dip coated sol-gel films onto fiber optics to form thinner coatings and techniques to enhance the fluorescence for improvements in optical signal and response times.

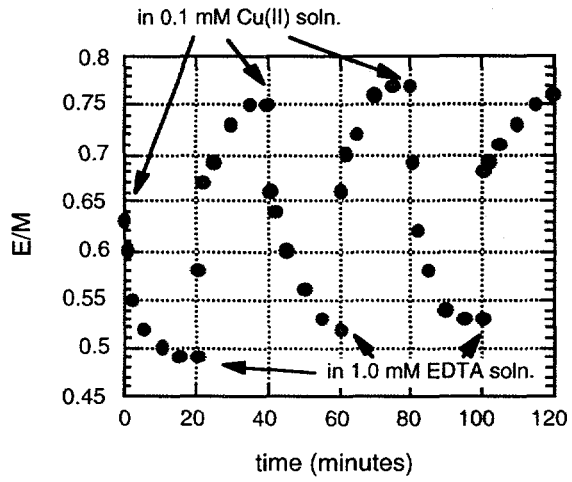


Figure 13. Cycling of $Cu^{2+}$ metal ion response and regeneration with EDTA of the fiber optic probe.

## 4. SUMMARY

Immobilized lipid-based sensor materials were successfully prepared as bulk and thin film materials using a TMOS sol-gel method. The sol-gel procedure quantitatively entrapped the bilayers and was highly compatible with the fragile self-assembled materials. Immobilization in the matrix provided protection from physical stress and biological predation. The silica matrix was also found to improve the sensor material's sensitivity and optical response to divalent metal ions. Although aging of the gel did create slight changes in the fluorescence output of the metal sensitive 5% PSIDA/DSPC lipid bilayers, the optical response to metal ions remained efficient and in most cases was enhanced over the response of bilayers in free solution. Enhancements in sensitivity of 4 – 100 fold were realized. Response curves also gave improved linear response to metal ions compared to bilayers in free solution; a possible result from the elimination of complications from light scattering due to bilayer aggregation in free solution. Thin film materials were coupled to fiber optic platforms to create a simple but effective metal ion sensor. Response times of minutes and equally rapid regeneration times are now possible. Further improvements of the sensor material, through thin film preparations and matrix modifications, are currently being explored.

## ACKNOWLEDGEMENTS

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